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HUMAN *BORDETELLA BRONCHISEPTICA* ISOLATES FROM HUNGARY

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Bordetella bronchiseptica is a widespread bacterial pathogen causing a number of respiratory diseases in mammals. Increasing number of human infections have been reported mostly in immunocompromised patients; occasionally the animal origins of the infections have also been established, therefore *B. bronchiseptica* is regarded as zoonotic agent. Based on latest genetic methods (e.g. MLST, genome sequencing), two distinct lineages have been distinguished within *B. bronchiseptica*. One of them is a human-adapted lineage which contains (mainly, but not only) human strains. The other, ancient lineage is animal-adapted. It includes strains from different animal host species and also a number of strains of human origin. Our previous study demonstrated that signs of host adaptation maybe observed among the strains, and host animal species (dog, cat, pig, etc.) can be determined by investigation of virulence genes. In this work, three human originated strains from Hungary were examined. Strain 1 was isolated in Budapest, 2014 from a 57-year-old, dog-owner female patient with deteriorating chronic obstructive pulmonary disease (COPD). Strain 2 was also isolated in Budapest, 2014 by pre-operative check-up from nasal secretion of a 82-year-old man who did not have any pet. Strain 3 was isolated in Szeged, 2007, but further details are unknown. The presence of four virulence factor genes (dermonecrotoxin [*dnt*], adenylate cyclase-haemolysin [*cya*A], fimbria [*fim*A] and flagellin [*fla*A]) as well as the *cya*A-replacement *ptp* gene was examined by polymerase chain reaction (PCR). PCR products of *cya*A, *fim*A, *fla*A and *ptp* were cleaved by BglI, HincII, NarI and/or SalI restriction endonucleases in restriction fragment length polymorphism (RFLP) method. On the basis of PCR-RFLP of *fim*A, the three human strains were uniform, but the results of the other genetic assays presented differences among the strains. Strain 2 and Strain 3 were identical: both strains were *fla*A- and *cya*A-positive and *dnt*- and *ptp*-negative. PCR-RFLP analysis of *fla*A- and *cya*A revealed the identity of these strains. According to our previous study, the flaA fragment pattern of Strain 2 and Strain 3 are not typical among any animal originated strain, and their *cya*A-type is equal to the *cya*A RFLP-type of the most foreign human strains. Based on these, Strain 2 and Strain 3 could be sorted into the human-adapted lineage of *B. bronchiseptica*.

On the other hand, Strain 1 had *dnt*, *fla*A and *ptp* genes, but did not possess the *cya*A gene. These properties are typical of Hungarian, dog originated *B. bronchiseptica* strains. In RFLP assays, the *fla*A and *ptp* fragment patterns of Strain 1 also showed identity with a profile of strains form dogs. Presumably, the patient carrying Strain 1 was infected by her dog.

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EPIDEMIOLOGY AND LABORATORY DIAGNOSTICS OF *BLASTOCYSTIS* SPECIES

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Blastocystis species are world-wide distributed enteral protozoons capable of parasitizing both animals and humans. Isolates from human samples can be categorised into 9 subtypes (ST) based on the sequence analysis of small subunit rDNA. Mainly ST1-ST4 and ST6 cause human disease. Due to its polymorphic nature, recognition and exact identification of the pathogen require substantial practice. The aims of this study were to optimize the traditional procedures (microscopic examinations and culturing), and to develop a sensitive, specific PCR-based molecular detection method. Based on the result of 100 tested stool samples we can conclude that direct microscopic examination – because of its low sensitivity - should be supplemented by at least one culturing method. For this purpose, the Boeck-Drbohlav-Locke medium containing serum and egg without vaseline is appropriate. For development of the molecular method, DNA was isolated from *Blastocystis*-positive stool samples. A PCR method based on the primers RD5 (5'-ATCTGGTTGATCCTGCCAGT-3') specific for eukaryotes and BhRDr (5'-GAGCTTTTTAACTGCAACAACG-3') specific for the genus *Blastocystis* was developed. Sequence analysis of the amplified fragments confirmed that this optimized process is a specific and sensitive protocol with the potential to be applied for routine laboratory diagnostics. The highest positivity rate (31%) was obtained by this molecular method compared to traditional examinations (3%). Among patients with positive samples, the youngest was 2 years old, and the mean age was 35 years. The positivity rate was the highest among patients between 20 and 40 years, but the difference was not significant between genders. The main symptom was abdominal pain, and this protozoon was detected in 3 patients suffering from colitis ulcerosa.

DETECTION OF *ASPERGILLUS* GALACTOMANNAN ANTIGEN IN HUMAN SAMPLES WITH DIFFERENT METHODS

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Fungal infections including several forms of aspergillosis have increased over the years among immunocompromised hosts. *Aspergillus* disseminates by means of conidia, which disperse readily throughout the environment because of their lightweight. Airborne conidia enter human host via inhalation or inoculation. Invasive pulmonary aspergillosis is the most severe form, but aspergilloma or allergic forms also may become invasive. As the microorganism is rarely recovered from sputum and may only be a contamination the diagnosis of a pulmonary infection is difficult to establish. Diagnosis may be facilitated by the use of serological examinations such as detection of the *Aspergillus* polysaccharide antigen (galactomannan) in serum or in bronchoalveolar lavage. In our laboratory two techniques have been used from 2004 to 2014. In this era 1655 galactomannan test were made. 66% of the positive samples was from male patiens and 34% was from female patiens. In the first six years a simple latex agglutination technique was used to detect galactomannan antigen in 986 cases. Only 13 tests (1.38%) were positive. In 2010 we introduced a new method: enzyme immunoassay. 669 samples have been tested from then and 35 (5.38%) were positive. In the examined decade both, cultivation from samples of respiratory tract and galactomannan antigen detection were made with samples from 369 patients. 19 of 44 galactomannan positive patients had positive cultivation (43%), but only three (7%) were *Aspergillus* sp. positives. 40 of 325 galactomannan negative patients had *Aspergillus* sp. positive cultivation. Only 0.7% of patients who